Therapeutic potential of inhibiting mitochondrial fission to reduce abdominal aortic aneurysms

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Abdominal aortic aneurysms (AAA) are a common and significant cause of premature death in the World. A greater understanding of the pathogenesis of AAA may enable the development of new pharmacological treatments to stabilise AAAs and to prevent rupture and future clinical symptoms. This would be highly beneficial, particularly in patients where the aneurysm is not severe enough to justify surgery. AAAs are characterised by localised structural deterioration of the artery wall, in particular thinning of the medial layer and degeneration of the internal elastic lamina. This results in progressive vessel dilatation and can lead to complications such as vessel rupture, which cause massive internal bleeding and death. Risk factors for aneurysm formation include male sex, age, family history, smoking and hypertension. Surgical interventions include endovascular stent grafting, or open surgical repair in which a graft is inlaid into the aneurysm sac, across the weakened area of the artery. Although these treatments are often very successful (2-5% mortality for elective surgery), mortality in patients with ruptured aneurysms remains high. Identification of patients with aneurysms through screening programmes may enable the application of novel treatments to reduce rupture and would therefore be a major breakthrough.

Mitochondrial fission is a stress response and is associated with apoptosis. Mitochondrial fission depends on dynamin related protein 1 (Drp1), a cytoplasmic large GTPase that induces fragmentation of mitochondria, peroxisomes, and endoplasmic reticulum. In an article within this issue *Cooper et al* investigated the role of mitochondrial fission in AAAs[1]. Interestingly, Drp1 was more abundant in both human AAAs and a mouse model of AAA (lysyl oxidase inhibitor β-aminopropionitrile plus Angiotensin-II infusion), which may lead to mitochondrial dysfunction and inflammation. The Drp1 inhibitor, mdivi-1, decreased Angiotensin-II induced mitochondrial fission within rat aortic VSMCs *in vitro* but did not affect untreated VSMCs. This study focused on VSMCs but since multiple cell types contribute to the pathogenesis of AAAs, it would be extremely interesting to extend this study to evaluate the effects of mdivi-1 in other relevant cell types such as macrophages and endothelial cells.

Localisation and post-translational modifications including phosphorylation, ubiquitylation and SUMOylation of Drp1 are critical determinants of its function. *Cooper et al* demonstrated Angiotensin-II treatment of VSMCs increased ERK1/2 dependent phosphorylation of Drp1-Ser616[1]. Future investigation using mitochondrial immunoprecipitation to assess the effects of Ang-II on Drp1 translocation to the mitochondria *in vitro* as well as the localisation of Drp1 and phospho-Drp1 within the aortic wall and within the aneurysm of human and mice AAA would corroborate the conclusions of *Cooper et al* and provide further insight into mitochondrial fission in AAAs. However, mdivi-1 may modify AAA disease via a range of different modifications of cell behaviour as a result of mitochondrial fission. For example Drp1 inhibition with mdivi-1 may affect contractility of the aorta since mdivi-1 inhibits phenylephrine-induced and endothelin-1-induced contraction of mouse and rat thoracic aortae[2, 3]. In addition, mdivi-1 retards calcification of cultured human aortic VSMCs[4], and therefore it may inhibit calcification within the aortae which is known to be associated with AAA progression. Further studies are necessary however, to demonstrate whether regulation of contraction and calcification due to mitochondrial fission contributes to AAA development.

The effects of Drp1 inhibition was assessed in two mice models of AAA: an atherogenic model (lysyl oxidase inhibitor β-aminopropionitrile plus Angiotensin II infusion) and a non-atherogenic model ($ApoE^{-/-}$ plus Angiotensin II infusion)[1]. In both models Drp1 inhibitor, mdivi-1, decreased the aortic diameter indicative of suppressed AAA progression. Further work with heterozygous Drp1 null mice confirmed that the decreased aortic diameter observed in mdivi-1 treated mice was specific to Drp1 inhibition. Interestingly, mdivi-1 also decreased macrophage infiltration, oxidative stress, ER stress, VSMC senescence, matrix-degrading metalloproteinas-2 activity

and increased survival in the atherogenic model and these effects were independent of hypertension.

Cooper et al have shown the role of Drp1 in mitochondrial fission and potential of Drp1 inhibitors as a novel treatment for retarding AAA development in these mouse models (Figure 1)[1]. AAAs are frequently asymptomatic in humans and not diagnosed until significant disease progression and dilation has occurred, therefore therapeutically it is important for future work to investigate the effects of inhibiting mitochondrial fission on cells pre-treated with Angiotensin-II *in vitro* and pre-existing AAA *in vivo*.

Previous published studies have indicated that the diabetes type II treatments, Glucagon-like peptide-1 (GLP-1) agonists and metformin, reduce AAA growth. It is possible that the underlying mechanism for this beneficial effect is in part through the inhibition of Drp1. Firstly, the GLP-1 agonist, lixisenatide, inhibited the growth of pre-existing AAA in rats subjected to the elastase plus calcium chloride model method of AAA induction[5]. GLP-1 increased phosphorylation of Drp1-Ser637, inhibiting Drp1 activity, in A7r5 cells (rat aortic VSMC cell line)[6]. The beneficial effects of lixisenatide on AAA growth may be mediated by the inhibition of Drp1 through phosphorylation of Ser637 and decreasing ERK-dependent activating phosphorylation of Drp1 Ser616 and thereby decreasing mitochondrial fission. Moreover, GLP-1 and inhibition of Drp1 via mdivi-1 both modified VSMC behaviour by decreasing PDGF-induced migration and proliferation, which is relevant to AAA pathogenesis[6]. Similarly, metformin treatment for type II diabetes, is associated with decreased AAA growth[7]. Metformin reduced the amount of Drp1 in the aorta of streptozotocin-induced diabetic mice[8]. In summary, it is possible that GLP-1 agonists and metformin used to treat diabetes type II cause the negative correlation observed between diabetes type II and AAA via suppression of mitochondrial fission mediated by Drp1 which contributes to AAA pathogenesis. Smoking is a dominant risk factor for AAA. *In vitro* cigarette smoke extract increased Drp1 protein and mitochondrial fission in human airway smooth muscle cells[9]. Whilst *in vivo,* cigarette smoke exposure increased mitochondria lesions, indicating mitochondrial damage, in the aortas of $ApoE^{-/-}$ mice[10]. Together this indicates that cigarette smoke can modify mitochondrial fission and Drp1 protein levels and thereby this may modify the behaviour of VSMCs and contribute to AAA pathogenesis. These associations with diabetes treatments and smoking strengthen the proposition that Drp1 inhibitors may prove to be beneficial for reduction of AAA progression and related deaths (Figure 1).

Figure legend

Figure 1: Schematic representation of the proposed involvement of Drp1 and mitochondrial fission in AAA and potential therapeutic options.

Angiotensin-II increases ERK1/2 dependent phosphorylation and activation of Drp1-Ser616 in VSMCs enhancing mitochondrial fission leading to alteration in cellular processes and resulting in AAA. Smoking may induce AAA disease by enhances Drp1 and mitochondrial fission. GLP-1 inhibits ERK1/2 and induces phosphorylation of Drp1-Ser637 retarding Drp1 activity and mitochondrial fission and suppressing AAA disease. *Inset*: In conclusion, Drp1 inhibition by mdivi-1 and diabetes treatments (GLP1 agonists and metformin) may be therapeutically beneficial in reducing AAA via modification of Drp1 activity and mitochondrial fission.

Servier Art images utilised in this figure.

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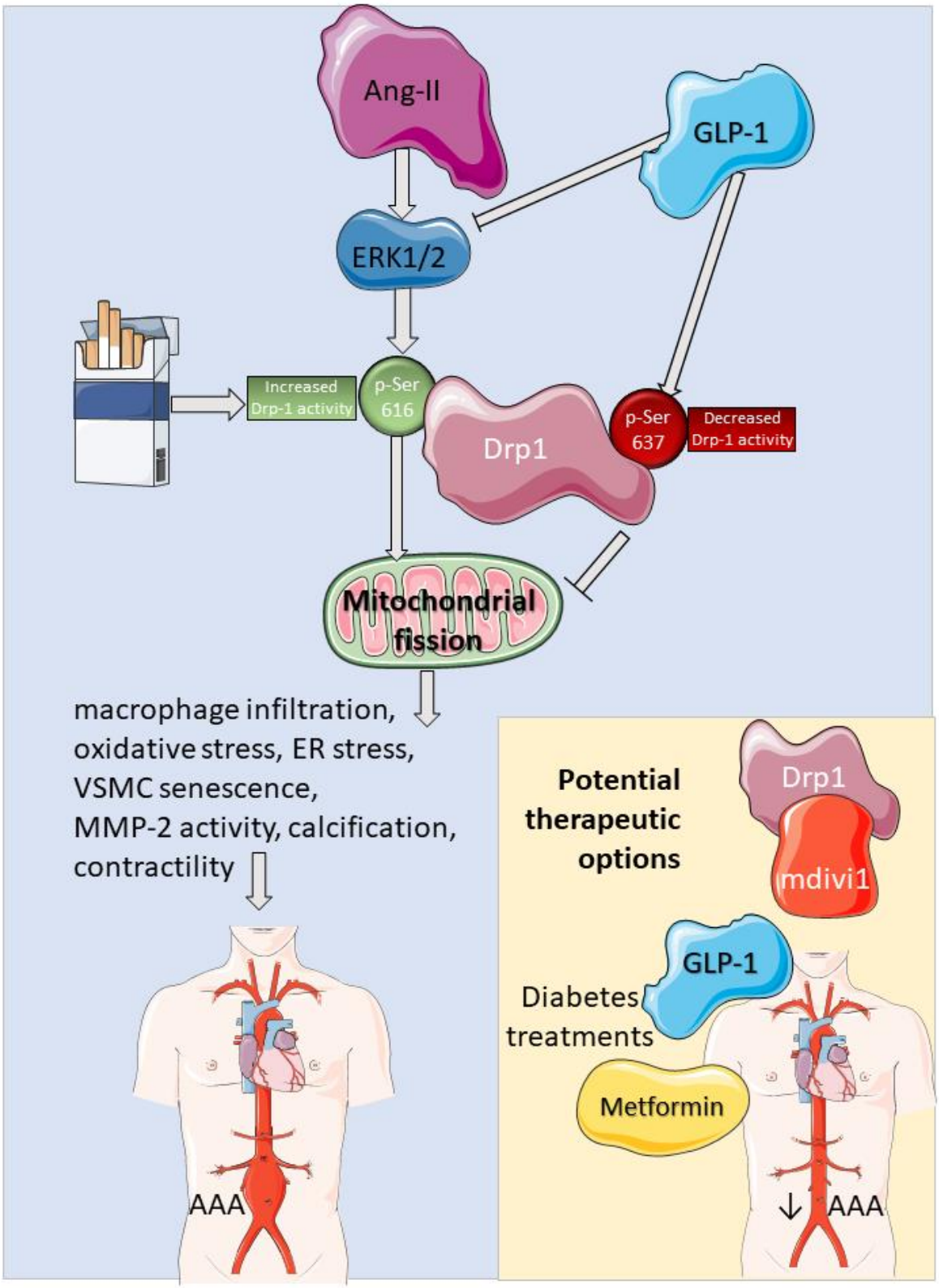

Graphical abstract